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Analysis of Coliphage Lambda Mutations That Affect *Q* Gene Activity: *puq*, *byp*, and *nin5*

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We describe in this paper the isolation and characterization of a class of mutations, designated *puq*, that allow phage λ to grow better under conditions that limit the synthesis of the phage *Q* gene product. These mutations were located between phage genes *P* and *Q*, a region of the λ chromosome containing two gene *N*-independent mutations, *nin5* and *byp*, that we also show to be *puq* mutations. Whereas the *puq*-3 and *puq*-16 mutations probably map under the *nin5* deletion, the *byp* mutation maps between this deletion and the *Q*λ-*Q*φ80 crossover point. These mutations likely act by increasing the synthesis of the *Q* gene product. We demonstrate that the clear-plaque phenotype and reduced lysogenization frequency of *byp* mutants depend on increased *Q* gene activity. The significance of these results in understanding how transcription proceeds through the *P*-*Q* region of the λ genome is discussed.

When coliphage λ infects *Escherichia coli*, one of two modes of phage development ensues. In the lysogenic mode, the phage coexists with the host without killing it. In the lytic mode, the phage rapidly reproduces and, in the process, kills the host. The lysogenic response is the result of repression of most phage functions by the phage *c*I gene product. The faithful segregation of repressed phage DNA (prophage) during cell division is ensured by integration of prophage DNA into the host genome, where it is replicated passively by the host. In contrast, during the lytic response, the phage DNA is actively replicated and transcribed, phage particles are assembled, and cell lysis occurs. In this report, we analyze the action of several phage functions involved in lytic growth.

Regulation of transcription during the lytic growth cycle depends on the action of the products of phage genes *N* and *Q* (16). In the absence of the *N* gene product, transcription that initiates at promoters to the left (p_L) and right (p_R) of the immunity region (Fig. 1) terminates at sites t_L and t_{R1}, respectively (20, 21, 27, 37). In the presence of the *N* gene product, transcription continues beyond these termination sites. By mutation, new promoters (e.g., *c*17 or ri^{c}_{5b}) have been isolated that initiate transcription to the right of t_{R1} (13, 23, 25, 26). This abnormal transcription terminates before reaching gene *Q* if the *N* gene product is absent, but continues into gene *Q* if the *N* gene product is present (9). This result suggested the existence of a second rightward termination site in addition to t_{R1}. This site, designated t_{R2}, was located between genes *P* and *Q* by analysis of two novel mutations, *nin5* (a 5.4% deletion) and *byp* (5, 8, 14, 19, 28). Phage carrying either one of these mutations no longer require the *N* gene product for transcription through t_{R2}, although such read through was enhanced in the presence of this product. Phage carrying the *nin5* deletion need no other mutations to grow in the absence of the *N* gene product, whereas the *c*17 promoter mutation must be included with the *byp* mutation to achieve *N* independence. Because the *N* gene product must also act at t_{R1} for normal synthesis of phage replication proteins, it is surprising that *nin5* phage grow in the absence of this product. The *nin5* mutation might eliminate this potential replication defect by either permitting more efficient utilization of the available replication proteins or increasing their synthesis.

The transcription of late phage genes (genes *S*, *R*, and *A* through *J* [Fig. 1]) is initiated primarily at p'_R and requires the *Q* gene protein (18). The mode of action of the *Q* gene product is unclear although it has been suggested that it acts like the *N* gene product in allowing transcription to continue beyond termination sites downstream from p'_R (28).

In this paper we describe the isolation, mapping, and characterization of mutations that al-

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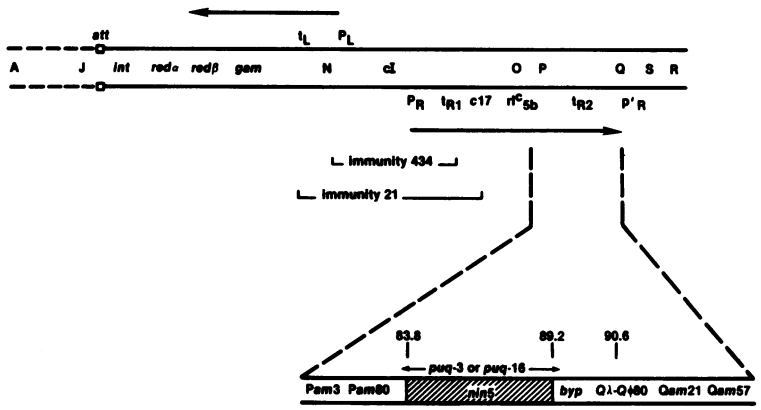


FIG. 1. *Genetic map of phage λ. Genetic distances are not drawn to scale, although the relative positions of the genes and sites are correct. The structural genes are placed between the two DNA strands, and the genetic sites are placed above and below the DNA strands. p_L, p_R, and p'_R refer to the phage promoter sites; t_L, t_{R1}, and t_{R2} refer to the phage terminator sites; c17 and ri^c_{5b} refer to new promoter mutations; and att refers to the phage attachment site. The two arrows indicate the direction of phage transcription during lytic growth. The region between phage genes P and Q is expanded with the λ map coordinates of the left and right ends of the nin5 deletion and the Qλ-Qφ80 crossover point shown (11).*

low the phage to grow better when the synthesis of the *Q* gene product is limiting. Included among these mutations are *puq* mutations, whose isolations are described in this paper, and the *nin*5 and *byp* mutations.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial and phage strains used in this paper are listed in Table 1.

Media. Tryptone broth contains 10 g of tryptone (Difco), 5 g of NaCl, and 5 ml of 1 M Tris per liter of water. TBMMB1 is tryptone broth supplemented with 0.4% maltose, 10 mM $MgSO_4$, and 1 μg of vitamin B_1 per ml. TB top and bottom agars are tryptone broth supplemented with 7 or 12 g of agar (Difco) per liter, respectively. Eosin methylene blue agar (EMBO) plates were prepared as previously described (30). TMG buffer contains 10 mM Tris-hydrochloride, pH 7.4, 10 mM $MgSO_4$, and 0.1% gelatin.

Standard phage procedures. Phage stocks were prepared from purified plaques by the plate lysate method (29). Single-step growth experiments (33) and phage crosses (32) were performed as previously described. When potential recombinant plaques for pertinent mutations were screened, the following phenotypes were used: (i) *cIts*857, clear plaques at 42°C; (ii) *byp*, clear plaques at 32°C and the ability to form plaques on strains NS377 and NS577 (33); (iii) *nin*5, the ability to form plaques on strains NS377 and NS577 and a decreased sensitivity of the phage to EDTA inactivation (24, 34); and (iv) *puq*, the ability to allow a *Qam* phage to form a plaque on strain CA169 (see below). Phage used to prepare extracts for the lysozyme assay were purified from plate lysates by banding in a CsCl equilibrium density gradient (34).

Measurement of lysozyme synthesis. A culture of strain W3350 growing in exponential phase at 10^8 cells per ml was concentrated by centrifugation (SS34 Sorvall rotor for 10 min at 6,000 rpm), and the pellet was suspended in 1/5 volume of a 10 mM $MgSO_4$ solution. A 2-ml aliquot of this cell suspension was infected with phage at a multiplicity of infection (MOI) of four phage per cell. After 5 min at 37°C for phage adsorption, the infected cells were diluted with 8 ml of tryptone broth, and the culture was grown with vigorous aeration at 37°C. Samples (1 ml) were removed at designated intervals, chilled to 4°C, and sonically treated with the fine probe of a Branson Sonifier (setting 3) for two 20-s intervals. The debris was removed by centrifugation (15 min at 6,000 rpm in an SS34 Sorvall rotor), and the supernatant was used to assay lysozyme as previously described (33). One unit of lysozyme is that amount of enzyme which produces 0.001 decrease in cell absorbance at 600 nm (A_{600}) in 1 min. The extract was adjusted so that the enzyme level used in each assay reduced cell absorbance 0.1 to 0.2 A_{600}/min. The background lysis of sensitized cells alone was 0.0005 A_{600}/min, and the activity is expressed as units per 100 μl of infected cells.

Measurement of lysogenization frequency. A culture of strain YMC or 594 growing in exponential phase at 10^8 cells per ml in TBMMB1 at 32°C was concentrated by centrifugation, and the pellet was suspended in 1/10 volume of a 10 mM $MgSO_4$ solution. The cells were starved for 30 min at 32°C, and then 0.1-ml samples (10^8 cells) were infected at an MOI of either 5 or 0.2 phage per infected cell. After 10 min for phage adsorption at 32°C, dilutions of these infected cells were spread on EMBO plates on which 10^8 *λh80(attλ-int)*$^{\Delta}$*c* selector phage had previously been spread. These plates were incubated overnight at 32°C, and colonies were scored as λ lysogens. As the infecting λ phage in these experiments carried the *cIts*857 mutation, we could independently confirm that these colonies were lysogens by their inability to grow at 42°C. The lysogenization frequency is the number of lysogens per number of infected cells, the latter being the cell titer at the moment of infection in the MOI = 5 experiment and 20% of this titer in the MOI = 0.2 experiment. We also measured the lysogenization frequency by spreading the infected cells on EMBO

TABLE 1. *Bacteria and phage strains*

Strain	Pertinent genotype	Comments/source/reference
Bacterial		
YMC	*supF*	12; used to assay λ*am* mutants
NS62	YMC(λ)	
NS617	YMC(λ*imm*434)	
NS61	YMC(λ*imm*21S*am*7)	
594	*sup*$^+$ *str*^{r}	6
W3350	*sup*$^+$	7
C600	*supE*	3
CA169	*supC*	4
NS377	*sup*$^+$ *nusA*-1 *rif*^{r}-2	33; used to assay λ*byp* and λ*nin*5 phage
NS577	NS377(φ80*psu*3$^+$)	33; used to assay λ*byp am* or λ*nin*5 *am* mutants
NS460	N215(*sup*$^+$) φ80^r	
N205	*recA*	35
SA268	λc*Its*857P*am*80$^+$(Q*am*501-*att*R)$^\Delta$	
SA431	λc*Its*857P*am*80$^+$(Q*am*501-*att*R)$^\Delta$	
SA613	λ(*att*L-P*am*80)$^\Delta$Q*am*21$^+$	
SA741	λ(*att*L-P*am*80)$^\Delta$Q*am*21$^+$	
SA307	λ(*att*L-P*am*80)$^\Delta$Q*am*21$^+$	
DC401	λ(*att*L-P*am*80)$^\Delta$*nin*5Q*am*21$^+$	
DC402	λ(*att*L-O*am*29)$^\Delta$P*am*3$^+$*nin*5Q*am*21$^+$	
Phage		
λc*Its*857Q*am*21		Phage strains were either obtained from the National Institutes of Health collection or were constructed by recombination between appropriate λ mutants, using selection procedures described in the text.
λc*Its*857Q*am*57		
λc*Its*857Q*am*501		
λcI$^+$Q*am*21		
λ*imm*21c*Its*S*am*7		
λc*Its*857*byp*		
λ*imm*434*byp*		
λc*Its*857O*am*29*byp*		
λc*Its*857*byp*Q*am*21		
λc*Its*857*nin*5		
λc*Its*857P*am*80*nin*5		
λc*Its*857*nin*5Q*am*21		
λc*Its*857*nin*5*byp*		
λc*Its*857*nin*5*byp*Q*am*21		
λc*Its*857P*am*80*nin*5*byp*		
λc*Its*857P*am*3*nin*5Q*am*21		
λc*Its*857*puq*-3 (or *puq*-16)Q*am*21		
λc*Its*857*puq*-3 (or *puq*-16)		
λcI$^+$*puq*-3 (or *puq*-16)Q*am*21		
λ*h*80*att*80*imm*434(*QSR*)φ80		
λ*h*80(*att*λ*int*)$^\Delta$*c*		

plates without selector phage and individually testing the resulting colonies for temperature sensitivity. The results obtained by the two methods were identical.

RESULTS

Isolation of *puq* mutants. We isolated mutations that specifically affect either the synthesis of the *Q* gene product or its activity as follows. λQ*am* mutants fail to form plaques on a host containing a *supC* ochre suppressor (strain CA169). Since these same phage form normal plaques on a host containing an amber suppressor (YMC*supF*) that inserts the same amino acid (tyrosine) at the amber site as the *supC* suppressor, the growth defect of λQ*am* mutants in strain CA169 is probably a consequence of their inability to make sufficient *Q* gene product. The levels of amber suppression in strains CA169 and YMC are, respectively, about 15 and 50% (15).

When λQ*am*21 is assayed on CA169, the efficiency of plaque formation is about 6×10^{-5} relative to that on YMC. Of these rare plaques, three classes can be distinguished on the basis of plaque size on CA169. The first class, representing about 10% of the plaque formers, contains phage that form normal-size plaques with the same efficiency on strains CA169, YMC, and 594 (*sup*$^+$). These phage are presumably amber$^+$ revertants. A second class of phage, also repre-

senting about 10% of the plaques, forms pinpoint plaques on strain CA169 and 594, but normal-size plaques on strain YMC. The efficiency of plaque formation is about the same in all cases. These mutants are probably the same as the "*Q*-independent (*qin*)" phage studied by Herskowitz and Signer (17) that exhibit an increased level of *Q*-independent transcription of late phage genes. These were not further characterized. The third class of phage mutants, representing the remaining 80% of the plaque formers on strain CA169, made normal-size plaques on YMC, small plaques with unit efficiency on CA169, and plaques with less than 10^{-5} efficiency on 594. Our hypothesis was that these phage acquired a second-site *puq* mutation, enabling them to grow better when either the synthesis or activity of the *Q* gene product was limiting. Since λ*puq*Q*am*21 phage do not form plaques on the *sup*$^+$ host, we suggest that at least some *Q* gene product is essential for improved phage growth. Three representative class 3 Q*am*21 pseudorevertants of independent origin (λ*puq*3Q*am*21, λ*puq*4Q*am*21, and λ*puq*16Q*am*21) were chosen for further analysis.

Specificity of *puq* mutations. The *puq* mutations might allow λQ*am*21 to grow better in strain CA169 because these mutations alter the *Q*-gene protein so that its activity is increased or because these mutations increase the level of *Q* gene expression. If the first explanation is correct, we might expect that suppression of the Q*am* growth defect by a particular *puq* mutation would be specific for the mutations chosen; that is, the protein alteration produced by the *puq* mutation might only increase the specific activity of the protein when tyrosine is inserted by an amber-suppressing tRNA at a particular location in the protein. If the second explanation is correct, *puq* suppression of the Q*am* defect should be independent of the particular Q*am* mutation used.

To test these two possibilities, we constructed phage by recombination, carrying each of three *puq* mutations with three different Q*am* mutations. We first selected Q*am*$^+$ revertants of each λ*puq*Q*am*21 phage by selecting normal-size plaques on strain 594(*sup*$^+$). The Q*am*$^+$*puq* mutants were then crossed with λQ*am*501 or λQ*am*57 in strain YMC(*supF*). The progeny of the crosses were plated on CA169. About 5% of the plaques were small (characteristic of Q*am* pseudorevertants) and, of these, about 20% were indistinguishable in their properties from λ*puq*Q*am* (see above). The Q*am* mutation could always be recovered from these λ*puq*Q*am* phage in backcrosses with wild-type λ by selecting phage that failed to form plaques on CA169 and 594 but plated normally on YMC. We obtained the same results with all three *puq* mutations, so nine combinations of λ*puq* Q*am* phage (three *puq* mutations and three Q*am* mutations) have the same properties. We suggest that the *puq* mutation increases the synthesis of the *Q* gene product and not the specific activity of the protein itself. Mapping data described in the next section place the *puq* mutations at a position to the left of the *Q* gene (Fig. 1), more definitively ruling out a direct alteration of the *Q* gene product by *puq*.

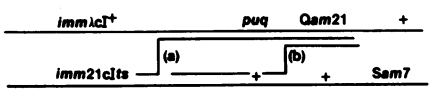
Mapping *puq* mutations. (i) Phage crosses between λ*imm*λc*I*$^+$*puq*Q*am*21 and λ*imm*21c*Its*S*am*7. We identified λ*imm*21c*Its*S*am*$^+$ recombinants among the progeny of this cross as clear-plaque formers on strain C600 at 42°C (Table 2A). A total of 75 to 90% of these recombinants carried the Q*am*21 mutation and, among these, 60 to 70% carried the *puq* mutation (recombinant class a). We conclude that the *puq* mutations tested (*puq*3, *puq*4, and *puq*16) lie between the right end of the *imm*21 substitution and the Q*am*21 mutations. Because most recombinants that carried the Q*am* mutation also carried the *puq* mutation, *puq* must be close to *Q*.

(ii) Crosses between λ*imm*λc*I*$^+$*puq*Q*am*21 and λc*Its*857 (*Q-att*R)$^\Delta$ prophage deletions. We tested a battery of prophage deletions that enter the λ prophage from the right and remove the *Q* gene, yet end within the region between genes *O* and *Q* (Table 2B). We infected these lysogens with the λ*imm*λc*I*$^+$*puq*Q*am*21 mutants and selected phage that rescued the c*Its*857 immunity marker from the prophage. Such phage form clear plaques on YMC at 42°C. If the prophage deletion contains the wild-type allele of *puq*, then some of the c*Its*857 recombinants should have lost the *puq* phenotype. Since none of the lysogens carry the prophage *Q* gene, the infecting phage cannot lose the Q*am* mutation. None of the recombinants tested (at least 100 from each cross) lost the *puq* mutation. As a control, we used λc*I*$^+$P*am*80 to infect either SA268 or SA431 and found that about 10% of the λc*Its*857 recombinant progeny were P*am*$^+$. We suggest that the wild-type allele(s) of the *puq* mutations is removed by the prophage deletions. These results and those shown in Table 2A place the *puq* mutations between the left end of the SA268 and SA431 deletions and the wild-type allele of the Q*am*21 mutation. We cannot rule out, however, that the *puq* mutations map outside and just to the left of the SA268 and SA431 deletions.

(iii) Crosses between λc*Its*857*puq*Q*am*21

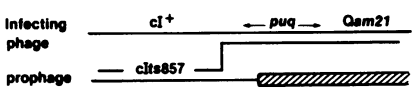
TABLE 2. *Mapping of puq mutations relative to the imm21 marker, the Q gene, and prophage deletions*[a]

A



puq mutation	*puq* recombinant fraction [(a)/(a) + (b)]
1	0.70
3	0.60
4	0.71
14	0.67
16	0.70

B



Prophage	*puq*+ phage among the λ*clts*857 Q*am*21 recombinants	
	puq-3	*puq*-16
λ*clts*8570*am*29+ (P*am*3-*att*R)Δ = SA439	0/120	0/123
λ*clts*857P*am*3+ (P*am*80-*att*R)Δ = SA444	0/100	0/110
λ*clts*857P*am*80+ (Q*am*21-*att*R)Δ = SA268	0/211	0/143
λ*clts*857P*am*80+ (Q*am*21-*att*R)Δ = SA431	0/140	0/126

[a] (A) λ*imm*21c*ltsSam*+ recombinants were selected as clear-plaque-forming phage on strain C600 at 42°C (the *Sam*7 mutation is not suppressed by the *supE* suppressor in strain C600). Eighty percent of these recombinants carry the Q*am*21 mutation as they are unable to form plaques on strain 594(*sup*+). We screened these λ*imm*21c*ltsQam*21 recombinants for the *puq* mutation based on their ability to form plaques on strain CA169. (B) We selected phage that had rescued the *clts*857 allele from the prophage as clear-plaque-forming phage on YMC at 42°C. These were screened for the *puq* mutation on strain CA169.

and λ*imm*434*bypQ*+. To position the *puq* mutations with respect to the *byp* mutation, we selected λ*imm*434Q*am*21 recombinant phage as turbid plaque formers on YMC(*sup*F) at 42°C (phage carrying the *byp* mutation form clear plaques [5]) (Fig. 2). The absence of the *byp* mutation among these recombinants was confirmed by their inability to form plaques on strain NS377 (see above and reference 33). Since the *byp* mutation is located between phage genes *P* and *Q* (see next section), the *puq* mutation might be located either to the right or to the left of *byp*. If *puq* were between *imm*434 and *byp* (Fig. 2, order 1), the fraction of λ*imm*434*puq*Q*am*21 phage among the recombinants would be a measure of the relative distances between *imm*434 and either *puq* or *byp*. In contrast, if *puq* were between *byp* and Q*am*21 (Fig. 2, order 2), we would expect all of the λ*imm*434Q*am* recombinants to carry the *puq* mutation since the formation of *puq*+ recombinants would require a triple recombination event. For *puq*3 and *puq*16, respectively, 12% (16/132) and 21% (22/106) of the λ*imm*434*byp*+Q*am*21 recombinants were *puq*+. Therefore, both *puq* mutations are to the left of *byp*, *puq*3 at a fractional distance 0.88 and *puq*16 at a fractional distance 0.79 of the distance between the right end of the *imm*434 substitution and the *byp* mutation. If we assume that these genetic distances are directly proportional to the physical distances between these markers, then the *puq* mutations can be located on the λ physical map. Since the λ map coordinate of the right end of the *imm*434 substitution is 79.1 (11) and the right-most coordinate of the *byp* mutation must be 90.6 (see below), we can locate the *puq*3 and *puq*16 mutations on the λ map between coordinates 88 and 89. This would put these *puq* mutations in the DNA sequences deleted by the *nin*5 deletion (DNA deleted between 83.6 and 89.2 on the λ map) or just to the right of that deletion (Fig. 1).

(iv) Crosses with λP*am*3*nin*5Q*am*21 and λ*puq*Q*am*57. To determine whether the *puq* mutations are indeed removed by the *nin*5 deletion, we crossed λP*am*3*nin*5Q*am*21 with λ*puq*Q*am*57 (Table 3). The Q*am*57 mutation is located to the right of Q*am*21 (18), and phage with this mutation will form plaques on YMC(*supF*) but not on C600(*supE*). Phage with

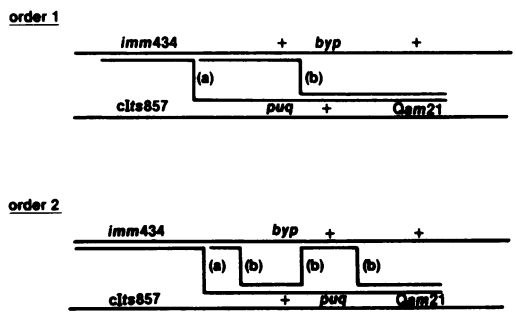
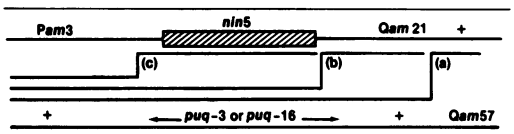


FIG. 2. *Turbid-plaque-forming recombinant phages (recombinants between the imm434 and byp markers) were selected on strain YMC at 42°C. Ninety-eight percent of these phage carried the Qam21 mutation as they were unable to form plaques on strain 594 (sup+). The presence of the puq mutation among these λimm434byp+ Qam21 recombinants was assessed by their ability to form plaques on strain CA169.*

the P*am*3 mutation will not form plaques on C600(*supE*) at 42°C. Thus, only Q*am*57$^+$ P*am*$^+$ recombinant phage will form plaques on C600 (*supE*) at 42°C. These recombinant phage were purified and tested for *nin*5, *puq*, and Q*am*21 as described in footnote *a* of Table 3 and above. An ambiguity arises in this analysis because it is not possible to distinguish between λ*nin*5Q*am*21 and λ*nin*5*puq*Q*am*21 because *nin*5 is a *puq* mutation (see next section). In addition, it is not possible to distinguish between λ*am*$^+$*puq* and λ*am*$^+$*puq*$^+$. In any case, we were unable to detect any Q*am*21*puq*$^+$*nin*$^+$ phage, suggesting that the *nin*5 deletion removes the wild-type alleles of *puq*3 and *puq*16. We cannot rule out the possibility that *puq* maps very close to but just outside of the *nin* deletion.

***byp* and *nin*5 are *puq* mutations.** The *puq*, *nin*5, and *byp* mutations are located in the same region of the λ genome. This proximity encouraged us to test *nin*5 and *byp* for the *puq* phenotype. To do this, the Q*am*21 mutation was introduced into λ*nin*5 and λ*byp*. λ*nin*5 and λ*byp* phage were crossed with λQ*am*21, and phage that formed small plaques on CA169 (about 2% of the yield from the cross) were selected for

TABLE 3. *Mapping of puq mutations relative to the nin5 deletion*[a]



puq mutation	No. of recombinants in class		
	(a) [*lam*$^+$]	(b) [*λpuqQam21*]	(c) [*λnin5* Qam21 or *λnin5 puqQam21*]
puq-3	10	20	35
puq-16	3	24	31

[a] The level of wild-type (*am*$^+$) recombinants was measured as plaque formers on strain 594(*sup*$^+$). λQ*am*21 recombinants were assayed on strain C600 at 42°C (neither λQ*am*57 nor λP*am*3 form plaques on C600 at this temperature) and represent 10% of the total phage burst. We determined whether the *nin*5 mutation was present in these λQ*am*21 recombinants by measuring either their ability to form plaques on strain NS577 or their EDTA sensitivity. Among the λQ*am*21 recombinants without the *nin*5 mutation, the presence or absence of the *puq* mutation could be assessed by the ability of these phage to form plaques on strain CA169(*supC*).

further analysis. The small-plaque phenotype itself strongly suggested that *nin*5 and *byp* were *puq* mutations. We demonstrated that these *Q* pseudorevertants were either λ*byp*Q*am*21 or λ*nin*5Q*am*21 by the following criteria: like both the *nin*5 and *byp* parents, the recombinants formed plaques on NS577 (33); the presumptive *nin*5 recombinant is as resistant to EDTA treatment as is the *nin*5 parent (wild-type λ is EDTA sensitive; deletions are EDTA resistant); and both phage do not form plaques on 594(*sup*$^+$) but do plaque on YMC(*supF*). Furthermore, they both fail to complement λQ*am*21 in 594(*sup*$^+$). Because these phage are able to grow on CA169, both *byp* and *nin*5 must be *puq* mutations. λ*byp*Q*am*21 forms larger plaques and λ*nin*5Q*am*21 forms smaller plaques than does λ*puq*Q*am*21 on CA169. The kinetics of phage production by these Q*am*21 phage in CA169 (see below) confirm this increasing ability to express the *puq* phenotype (*byp* > *puq* > *nin*5).

Mapping *nin*5 and *byp* mutations. Although both *nin*5 and *byp* mutations have been located in the interval between phage genes *P* and *Q*, their location relative to each other is unclear. (Are they at the same site? Is *byp* to the left or right of *nin*5?) The following three crosses locate more precisely these two mutations.

(i) Crosses between either λP*am*80*nin*5 or λO*am*29*byp* and λc*Its*857 (*Q-att*R)$^\Delta$ prophage deletions. We infected each prophage deletion with λP*am*80*nin*5 or λO*am*29*byp* and isolated amber$^+$ recombinants from the progeny by plating the phage yield on strain 594(*sup*$^+$) (Fig. 3A). These amber$^+$ plaques were then screened for loss of *nin*5 or *byp*. We detected no *nin*$^+$ or *byp*$^+$ plaques among several hundred amber$^+$ recombinants. We conclude that the two prophage deletions contained in strains SA268 and SA431 carry neither the wild-type allele of the *byp* mutation nor DNA to the right of the *nin*5 deletion.

(ii) Crosses between λ*nin*5Q*am*21 or λ*byp*Q*am*21 and (*attL-P*)$^\Delta$ prophage deletions. We used another set of prophage deletions that enter the *P-Q* region from the left (Fig. 3B). These strains were infected with λ*nin*5Q*am*21 or λ*byp*Q*am*21. Amber$^+$ recombinants were selected by plating the phage yield on 594 (*sup*$^+$), and phage in the resulting plaques were then tested for the loss of the *nin*5 or *byp* mutations. Among several hundred recombinant plaques obtained from the three crosses, none contained *nin*$^+$ phage. Similarly, *byp*$^+$ phage were not found in crosses involving SA613 or SA741. However, 20% of the amber$^+$ phage from

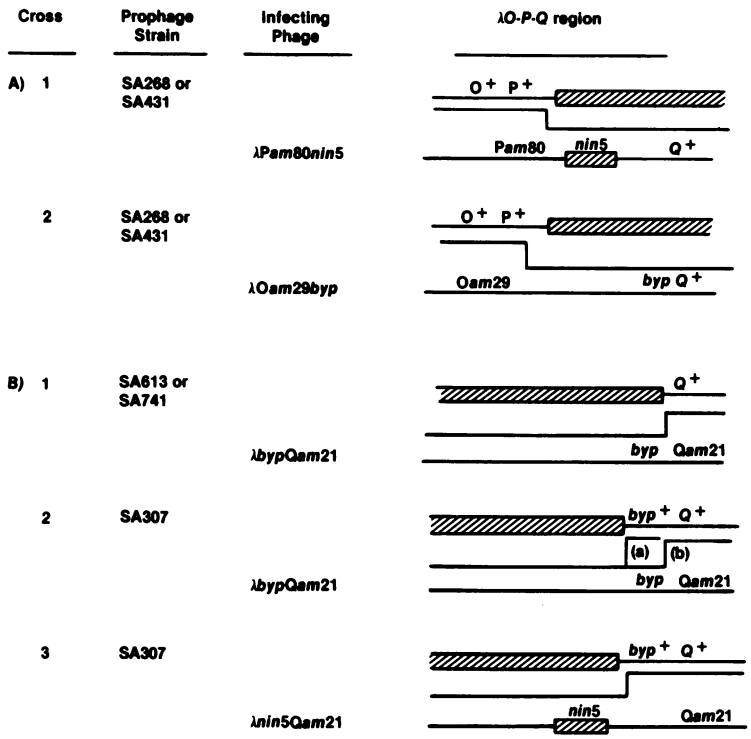


FIG. 3. *Recombinant phage in these crosses between λOam, λPam, and λQam phage and deleted λ prophages were selected as plaque formers on strain 594(sup⁺). Recombinants lacking the nin5 deletion (crosses A1 and B3) were detected by their inability to form plaques on strain NS377, and those lacking the byp mutation (crosses A2, B1, and B2) were detected by their turbid plaque phenotype and confirmed by their inability to form plaques on strain NS377.*

the λ*byp*Q*am*21 × SA307 cross were *byp*⁺ (cross 2 recombinant class a). SA307, therefore, contains the wild-type allele of *byp* but not DNA to the left on the *nin*5 deletion. We conclude that the *byp* mutation lies between the left end point of the *nin*5 deletion and gene *Q*.

(iii) Crosses with λc*Its*857*byp* and (*att*L-O*am*29)$^{\Delta}$ or (*att*L-P*am*80)$^{\Delta}$ cryptic prophage deletions derived from a λ*nin*5 prophage. To determine whether the *nin*5 deletion removes the wild-type allele of *byp*, we crossed λc*Its*857*byp* with prophage deletions derived from a λ*nin*5 prophage. (Fig. 4). In both cases, *byp*⁺ recombinants were detected. Two of four *byp*⁺ recombinants from the DC401 cross and three of eight *byp*⁺ recombinants from the DC402 cross were more sensitive to EDTA treatment than were *nin*5 phage and failed to form plaques on strain NS577, both expected characteristics of *byp*⁺ *nin*⁺ phage (recombinant class b). If the *byp*⁺ allele were located in a region of DNA removed by the *nin*5 deletion, then all *byp*⁺ recombinants in these crosses should be *nin*5. As this is not the case, the *byp*⁺ allele is not covered by the *nin*5 deletion. Based on these data and those presented above, we conclude that the *byp* mutation is located between the right end of the *nin*5 deletion and the *Q* gene.

Isolation of λ*nin*5*byp* double mutants. If *nin*5 and *byp* are at different sites, it should be possible to isolate the *nin*5*byp* double mutant. We did this by treating the progeny of the crosses shown in Fig. 4 with EDTA to inactivate *nin*⁺ phage. The surviving phage were then plated on 594(*sup*⁺). Ten to twenty percent of the resulting plaques were clear and thus were potential λ*nin*5*byp* phage. To verify their genotype, we isolated both *nin*5 and *byp* single mutants from the double mutant. The experiments were as follows. We first crossed either P*am*80 or Q*am*21 mutations into the presumptive λ*nin*5*byp* phage and then crossed the λP*am*80*nin*5*byp* phage with λQ*am*21 and the λ*nin*5*byp*Q*am*21 phage with λP*am*80 (Table 4). In both cases amber⁺ recombinants were selected and clear (*byp*) or turbid (*byp*⁺) plaques were tested for the *nin*5 mutation. We found that: (i) in cross 1, 30% of the turbid plaques

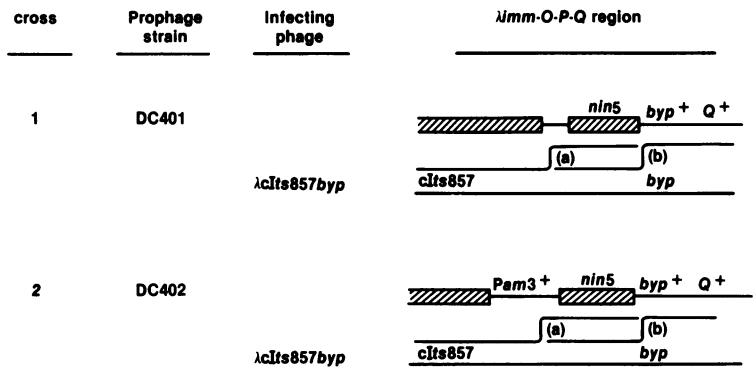


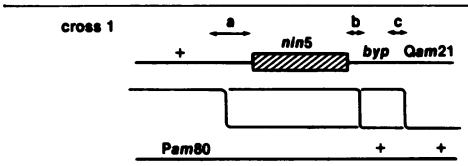
FIG. 4. *Recombinant phages with the byp⁺ allele were selected as turbid plaque formers on strain 594 at 42°C. The EDTA sensitivity of the phage contained in these plaques was tested under conditions in which nin5 phage are completely resistant and nin⁺ phage are inactivated to a level of 10^{-2} survival (EDTA treatment for 10 min at 32°C).*

contained *nin5byp*⁺ phage, and in cross 2, 35% of the clear plaques contained *nin*⁺ *byp* phage (in both cases, recombination occurred in segment b; therefore, the original phage must have carried the *nin*5 and the *byp* mutations); (ii) the location of *byp* between *nin*5 and Q*am*21 is confirmed because all of the clear-plaque formers in cross 1 were *nin*5, and all of the turbid-plaque formers in cross 2 were *nin*⁺ (recombination in both crosses is in segment c); (iii) the relative recombination frequencies in intervals a, b, and c are a measure of the relative lengths of the intervals (based on the results given in Table 4, the values for these distances expressed as a fraction of the total recombination distance between P*am*80 and Q*am*21 are: segment a, 0.44 to 0.49; segment b, 0.21 to 0.24; and segment c, 0.30 to 0.32).

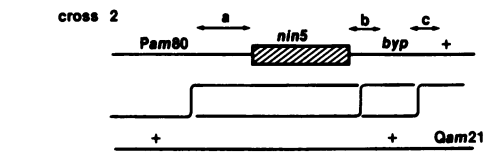
Mapping *byp* relative to the λ-φ80 recombination site in gene *Q*. A unique site for recombination between phages λ and φ80 is located within the *Q* gene of these phages at position 90.6 on the λ map (11, 14). As this site is immediately to the right of the *nin*5 deletion, we decided to use a λ-φ80 hybrid phage with λ DNA to the left of this recombination site and φ80 DNA to the right of this site to localize more specifically the *byp* mutation. We infected strain YMC with this hybrid phage and with λ*byp*Q*am*21 (Fig. 5) and selected *h*λQ*am*⁺ recombinant phage from the phage yield as plaque formers on a φ80-resistant derivitive of strain N215. Three of 1,120 recombinant plaques were clear at 32°C. Presumably, they contained phage with the *byp* mutation and were formed by recombination event c. The *byp*Qφ80 genotype of these recombinant phage was confirmed by the following tests: (i) they formed plaques on NS577, a property of *byp* mutants; and (ii) when an N205(*recA*) lysogen containing the presumptive λ*byp*Qφ80 prophage was infected with λ*red*3*imm*434R*am*5, plaques appeared at an efficiency of $< 10^{-4}$ relative to that found after infection of an N205(*recA*) lysogen carrying a λ*byp*Qλ prophage. Plaque formation by heteroimmune R^- phage on lambda lysogens of N205(*recA*) is an indication of the transactivation of the prophage *R* gene by gp*Q* of the infecting phage (36). The failure to transactivate the *R* gene from the presumptive *byp*Qφ80 prophage implies that it indeed carried φ80 DNA to the right of the λ-φ80 recombination site. Transactivation of the φ80*R* gene is not stimulated by the *Q* gene product of phage λ (10, 36). We conclude that *byp* is located between the right end of the *nin*5 deletion (89.2 on the map) and the Qλ-Qφ80 recombination site (90.6 on the λ map).

Single-step growth experiments. Up to now, we have discussed the *puq* phenotype only in terms of plaque size and plating efficiency. In this section, we quantitatively analyze the effect of various *puq* mutations on kinetics of phage development and yield of phage carrying Q*am* mutations. We used strains 594(*sup*⁺), CA169(*supC*), and YMC(*supF*) and infected them with wild-type λ, λQ*am*21, λ*byp*Q*am*21, λ*nin*5Q*am*21, λ*puq*3Q*am*21, and λ*nin*5-*byp*Q*am*21 (Fig. 6). In YMC(*supF*), all phages grew well, although λ*nin*5Q*am*21 seemed to give a slightly reduced phage burst. The *puq* phenotype is expressed in strain CA169(*supC*). In this host, λ*byp*Q*am*21 and λ*nin*5*byp*Q*am*21 grew almost as well as λ wild type. However, the growth of λ*puq*3Q*am*21, λ*nin*5Q*am*21, and λQ*am*21 phages was increasingly more defective both in the kinetics of appearance and in the yield of phage. In the *sup*⁺ host, only the wild-type

TABLE 4. *Ordering of Pam80, nin5, byp, and Qam21 mutations by four-factor crosses*[a]



amber⁺ recombinant	No. isolated	Recombinant frequency	Segment in which recombination event occured
byp nin⁺	0	--	--
byp nin5	17	0.30	(c)
byp⁺ *nin*⁺	28	0.49	(a)
byp⁺ *nin5*	12	0.21	(b)



amber⁺ recombinant	No. isolated	Recombinant frequency	Segment in which recombination event occured
byp nin⁺	24	0.24	(b)
byp nin5	44	0.44	(a)
byp⁺ *nin*⁺	32	0.32	(c)
byp⁺ *nin5*	0	--	--

[a] (Cross 1) Wild-type (*am*⁺) recombinants were assayed by plaque formation on strain 594 and represented 2% of the phage yield. Thirty percent of these plaques were clear (*byp*), and of 17 clear plaques tested all contained phage with the *nin5* mutation by virtue of their increased EDTA resistance. Among the remaining 70% turbid plaques, 12 of 40 tested were judged to be *nin5* by virtue of both their increased EDTA resistance and their ability to form plaques on strain NS377. (Cross 2) In this cross 2.8% of the phage yield were *am*⁺ recombinants and of these, 32% were turbid (*byp*⁺) and 68% were clear (*byp*). The presence of the *nin* allele among these recombinants was assessed as in cross 1.

phage grew normally. λ*nin5byp*Q*am*21 and λ*byp*Q*am*21 produced only two- to fourfold more phage than did other λQ*am*21 phage in this host. Whereas λ*byp*Q*am*21 and λ*nin5*Q*am*21 phage did not form plaques on 594(*sup*⁺) [less than 10^{-5} relative to plaque formation on YMC(*supF*)], λ*nin5byp*Q*am*21 phage formed pinpoint plaques on 594(*sup*⁺) with almost wild-type efficiency.

Kinetics of endolysin production. The efficiency of activation of late phage genes by the *Q* gene product is generally assayed by measurement of phage endolysin (the phage *R* gene product) (9, 22, 33). We measured endolysin after infection of strains YMC(*supF*), CA169(*supC*), and 594(*sup*⁺) by λQ*am*21 and λQ*am*⁺ phages (Fig. 7). In YMC(*supF*), all phages produced normal or near-normal amounts of endolysin activity, ranging from 800 to 1,000 units by 40 min after infection (Fig. 7a). After infection of CA169(*supC*) with λQ*am*21, endolysin production was much reduced relative

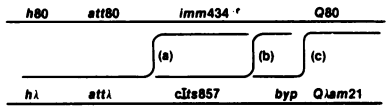


FIG. 5. λ*byp*Q*φ80 recombinant phage were detected as clear-plaque-forming phage on strain NS460 at 32°C. We could confirm that these clear plaques contained phage with the byp mutation as these phage formed plaques on strain NS377.*

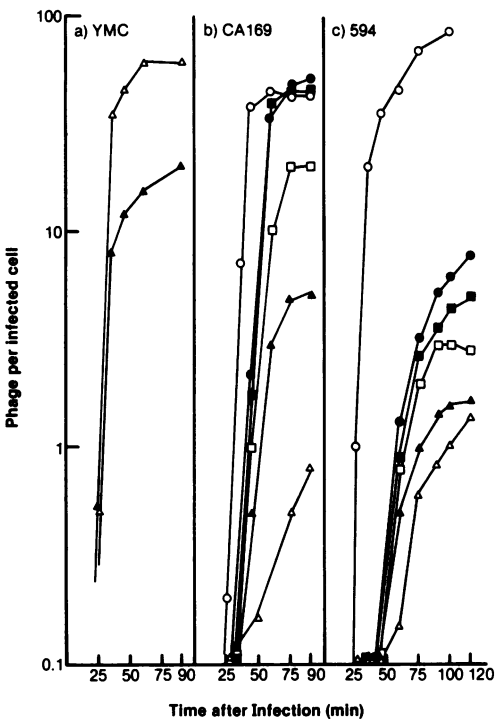


FIG. 6. *These single-step growth experiments were performed as previously described (33). The phage yield was sampled at designated times after infection by blending samples of the infected cell culture in a Vortex mixer with several drops of chloroform. The bacterial strains used were YMC (a), CA169 (b), and 594 (c). The phage used were* λ*cIts857Qam*⁺ (○), λ*cIts857Qam21* (△), λ*cIts857nin5Qam21* (▲), λ*cIts857puq-3Qam21* (□), λ*cIts857bypQam21* (■), *and* λ*cIts857nin5bypQam21* (●).

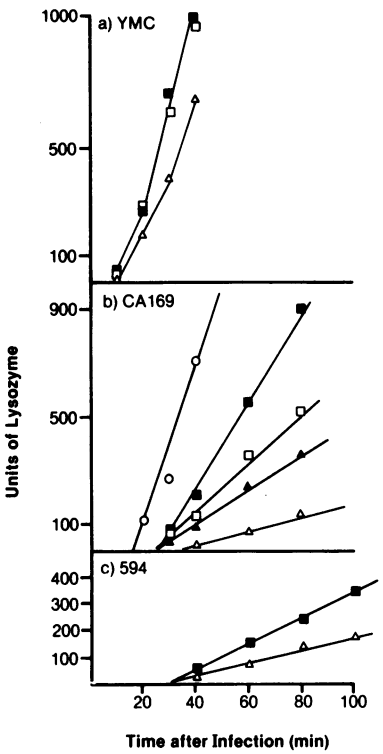


FIG. 7. *Lysozyme assays were performed as described in the text. The bacterial strains used were YMC (a), CA169 (b), and 594 (c). The symbols for phage used were the same as those given in the legend to Fig. 6.*

to the YMC(*supF*) infection. The *nin*5, *puq*3, and *byp* derivatives of λQ*am*21 produced, respectively, increasing amounts of endolysin after infection of CA169(*supC*) (Fig. 7b). In contrast, endolysin production by both λQ*am*21 and λ*byp*Q*am*21 in 594(*sup*$^+$) was much reduced; the *byp* mutation enhanced enzyme production by only a factor of 2 (Fig. 7c). Comparing the results shown in Fig. 7b and c, we conclude that the *byp* mutation produces a twofold increase in endolysin synthesis in the absence of the *Q* gene product (λQ*am*21 in strain 594) and a more than sixfold increase in endolysin synthesis when synthesis of the *Q* gene product is limiting (λQ*am*21 in strain CA169).

Clear-plaque phenotype of λ*byp* phage. Phage that carry the *byp* mutation form clear plaques and are impaired in their ability to form lysogens. The frequency of lysogen formation was generally three- to sixfold less than that found for *byp*$^+$ phage (Table 5). We have shown in the two previous sections that the *byp* mutation increases the expression of the *Q* gene and late phage functions. This increased expression could channel a phage infection into a lytic rather than lysogenic response. We would expect that if this were so, λ*byp*Q*am*21 phage would form lysogens with a higher frequency than λ*byp*Q$^+$ phage in YMC(*supF*) and with even a higher frequency in 594(*sup*$^+$). This is, in fact, what we observed (Table 5). In YMC(*supF*) and 594(*sup*$^+$) hosts, the Q*am*21 mutation increased the frequency of lysogen formation by λ*byp* phage, respectively, two- and fourfold. Indeed, λ*byp*Q*am*21 forms a more turbid plaque on YMC(*supF*) than does λ*byp*.

DISCUSSION

In this paper we have described the isolation and characterization of mutations (designated *puq*) in phage λ that increase synthesis of the *Q* gene product. Normally, these mutations have no phenotype because the level of the *Q* gene product synthesized by wild-type λ or by λQ*am* phage grown in strain YMC(*supF*) is more than sufficient for phage production. However, if λQ*am* phage are grown in the weak *am*-suppressing strain CA169 (under these conditions, synthesis of the *Q* gene product is limiting for phage growth), then the *puq* mutation helps to alleviate the phage growth defect. Thus, whereas λQ*am*21 fails to plaque on strain CA169, λ*puq*Q*am*21 plaques with unit efficiency on this host. Since *puq*Q*am* phage do not grow in strains lacking either *am* or *oc* suppressors, at least some synthesis of the *Q* gene product is necessary for *puq* to exert any affect on λQ*am* plaque formation; *puq* does not create a *Q*-independent phenotype, as do mutations previously described by Herskowitz and Signer (17). Various *puq* mutations have been mapped between phage genes *P* and *Q*, a region of the genome known to contain two mutations (*nin*5 and *byp*) that allow λ to grow better in the absence of the phage gene *N* product. Both *byp* and *nin*5 are also *puq* mutations. Based on plaque size, phage burst, and phage endolysin synthesis, *byp* is a better

TABLE 5. *Lysogenization frequency of λbyp phage*[a]

Infecting phage	Bacterial strain	Lysogenization frequency at: MOI = 5	MOI = 0.2
λc*Its*857	594(*sup*$^+$)	0.4	0.5
λc*Its*857Q*am*21	594(*sup*$^+$)	0.25	0.36
λc*Its*857*byp*	594(*sup*$^+$)	0.08	0.12
λc*Its*857*byp*Q*am*21	594(*sup*$^+$)	0.30	0.40
λc*Its*857	YMC(*supF*)	0.5	0.25
λc*Its*857Q*am*21	YMC(*supF*)	0.25	0.30
λc*Its*857*byp*	YMC(*supF*)	0.10	0.06
λc*Its*857*byp*Q*am*21	YMC(*supF*)	0.17	0.12

[a] Lysogenization was measured as described in the text.

puq mutation and *nin*5 is a worse *puq* mutation than are the mutations (*puq*-3 and *puq*-16) whose isolations are described in this paper.

A map of the λ genome, with particular focus on the region between phage genes *P* and *Q*, is shown in Fig. 1. The map coordinates of the *nin* deletion are well documented (11). Crosses described in Results located the *puq* mutations either under the *nin* deletion or very close to the left or right end of this deletion. The *byp* mutation was localized to the right of the *puq* mutations and the *nin* deletion (right map coordinate 89.2) and to the left of the crossover point in the *Q* genes of phage λ and phage 80 (map coordinate 90.6). We have isolated λ*nin*5*byp* double mutants, confirming the location of *byp*.

Based on our analysis of the properties of *nin*, *byp*, and *puq* mutations, we can draw the following conclusions about the regulation of *Q* gene expression and the activity of the *Q* gene product.

(i) The *byp* mutation increases the level of phage endolysin synthesis by a factor of no more than 2 when the *Q* gene product is not synthesized (Fig. 7). Transcription of the phage endolysin gene (gene *R*) in the absence of the *Q* gene product initiates exclusively at p_L and reads through into genes *Q* and *R* (9). Since *R* gene transcription is enhanced twofold by the *byp* mutation, *Q* gene transcription should be enhanced to the same extent. Yet, when a limiting amount of *Q* gene product is synthesized (Fig. 7b), the enhancement of endolysin synthesis by *byp* is in excess of sixfold. These results indicate that the concentration dependence of the activity of the *Q* gene product is not linear. This might be the case if the product of gene *Q* is required as a dimer or a trimer. In such a circumstance, a twofold reduction in monomer synthesis might mean a fourfold reduction or even an eightfold reduction in activity.

(ii) λ*byp* phage form clear plaques and lysogenize standard bacterial hosts with a three- to sixfold-reduced frequency relative to *byp*$^+$ phage. In contrast, λ*byp*Q*am* phage lysogenize strain 594(*sup*$^+$) at the same frequency as do λ*byp*$^+$ phage and lysogenize strain YMC(*supF*) at a frequency intermediate between λ*byp* and λ*byp*$^+$ phage (Table 5). These results suggest that after infection the lysogenization defect of λ*byp* phage reflects an increased expression of the *Q* gene and late phage functions, with a consequent channeling of the infection into a lytic rather than the lysogenic response. If the synthesis of *Q* gene product is reduced or eliminated, then the lysogenization frequency of *byp* phage is the same as that of *byp*$^+$ phages.

(iii) How do *nin*5, *byp*, and *puq* mutations exert their affects on gp*Q* activity? We can rule out the possibility that these mutations directly alter the *Q* gene protein because *nin*5 is a deletion that maps to the left of the *Q* gene, and the *puq* phenotype exhibited by *puq*-3, *puq*-16, and *byp* is independent of the Q*am* mutation used (see Results; data for *byp* is not shown). More likely, these mutations act by increasing *Q* gene transcription. As we cannot detect endolysin production from λ*puq*-3, λ*puq*-16, λ*nin*5, and λ*byp* lysogens (data not shown; also see reference 5), these mutations must not create a constitutive *Q* and *R* gene promoter like the *qin*101 mutation (10). Since the region between phage genes *P* and *Q* contains a transcription termination site (t_{R2}) that is sensitive to the *N* gene product, the *puq*, *nin*5, and *byp* mutations might alter or eliminate this site so that transcription beyond it becomes *N* independent. If transcription antitermination mediated by the *N* gene product were less than 100% efficient at t_{R2}, then these mutations would enhance transcription of downstream genes (*Q* and *R*) even in the presence of the *N* gene product. Indeed, transcription stimulated by this product decreases as the number of termination sites traversed by the polymerase increases (1, 2), suggesting that *N*-mediated transcription antitermination is not 100% efficient at each termination site.

Although this model fits well with the nature of the *nin* mutation, a deletion which could eliminate the t_{R2} termination site, it does not readily explain some of the properties of the *puq* and *byp* mutations. First, whereas *nin*5 and *byp* mutations are *N*-independent mutations, *puq*-3 and *puq*-16 are not [λN*am*7N*am*53*c*17*byp* and λN*am*7N*am*53*nin*5 plaque on strain 594(*sup*$^+$), but λN*am*7N*am*53*c*17*puq* does not (data not shown)]. Yet, *puq*-3 and *puq*-16 mutations show a stronger *puq* phenotype than does the *nin*5 mutation (Fig. 6 and 7). These discrepancies are most easily reconciled if the *puq* mutation does not affect a termination site but rather creates a promoter upstream from t_{R2}. Thus, its ability to stimulate *Q* gene transcription would still depend on *N* product activity. Second, since *byp* is not covered by the *nin*5 deletion, if *nin*5 deletes t_{R2}, then *byp* must either affect a second transcription termination site in this region or must create a new promoter capable of transcribing the *Q* gene in the absence of the *N* gene product. As such a promoter is not active in the prophage state (5), we suggest that it requires upstream transcription (e.g., from p_R) for its activation. In the absence of the *N* gene product, the low level of transcription that might proceed through the t_{R2} site could activate this new promoter, resulting in an increased *Q* gene tran-

scription.

In addition to the *nin*5, *byp*, and *puq* mutations, a fourth mutation which maps in the t_{R2} region of the λ map is *pasB* (31). This mutation enables a λ*red*$^-$ *gam*$^-$ phage to grow better in strains containing a *polA* mutation (the *feb* phenotype [38]). Preliminary experiments indicate that *nin*5 and *puq*-3 are *pasB* mutations but that *byp* is not. As the *pasB* phenotype is not well understood, the significance of these findings must await further experimentation.

An analysis of the transcription patterns in the *P-Q* region of the λ map produced by the various mutant phages should help to elucidate their mode of action.

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